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EFFECT OF 17β-ESTRADIOL ON THE ACTIVITY OF Δ⁵-3β-HYDROXYSTEROID DEHYDROGENASE IN THE TADPOLE LIVER

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ABSTRACT

Chih-Yün Hsü and Liü-Hsin Hsü (1976). *Effect of 17β-Estradiol on the Activity of Δ⁵-3β-Hydroxysteroid Dehydrogenase in the Tadpole Liver.* Bull. Inst. Zool., Academia Sinica **15**(1): 15-19. Tadpoles of *Rana catesbeiana* were reared in water containing 17β-estradiol (2 mg/l). The tadpoles were sacrificed after 7 months of treatment. Their livers were examined histochemically and biochemically for the effect of estradiol on the activity of Δ⁵-3β-hydroxysteroid dehydrogenase. The results from both methods ran closely parallel, showing reduced hepatic enzyme activity of the treated tadpoles when compared with that of the control animals. The finding was discussed from the functional point of view of the liver to contribute Δ⁵-3β-HSD for the synthesis of bile acids and possible metabolism of steroid hormones.

Δ⁵-3β-hydroxysteroid dehydrogenase (Δ⁵-3β-HSD) is generally known as the key enzyme in the biosynthesis of steroid hormones of the C_{19} and C_{21} series. Therefore, the enzyme is distributed primarily in steroidogenic endocrine tissues in various vertebrate classes.

The liver is the largest gland in the body, serving multiple functions. Among a host of enzymes which the organ harbors, Δ⁵-3β-HSD has been identified in rats[3,4,10,14]. It was shown that Δ⁵-3β-HSD in the rat liver plays a role in the synthesis of bile acids from cholesterol where C_{27} steroid is involved[3,4]. In addition to its nonendocrinological role, the hepatic enzyme may also contribute to the peripheral conversion of dehydroepiandrosterone (DHEA) and pregnenolone to their respective immediate metabolites[6,14,20].

In the present study, Δ⁵-3β-HSD was demonstrated too in the tadpole liver by histochemical and biochemical methods. It was further indicated that 17β-estradiol (E_2) depressed the activity of Δ⁵-3β-HSD in the tadpole liver which conformed to our earlier report that the enzyme activity in tadpole interrenals and ovaries was decreased by the same hormone[17].

MATERIALS AND METHODS

Tadpoles of *Rana catesbeiana* at the metamorphic stage III[29] with average body weight of 0.9 gm were reared in dechlorinated tap water containing 17β-estradiol* in the concentration of 2 mg per liter of water. Tadpoles raised in plain water served as the controls. The aquarial water was changed 3 times weekly and the hormone was added anew. The tadpoles were fed thawed

* All biochemicals were bought from Sigma except radioactive pregnenolone and polyvinyl alcohol.

leaves of water convolvulus and maintained at a water temperature of 20±1C with 10 hours of incandescent light each day beginning at 8 AM.

After 7 months of treatment, the 2 groups of tadpoles were killed; the body weight of the tadpoles was measured individually. Their livers were flesh-frozen on dry ice and stored in deep freezer at −70C.

Eight μ frozen sections of the tadpole livers were prepared with an AO cryocut at −25C. They were incubated for 2 hours at 37C in the medium containing DHEA as the substrate, NAD (nicotinamide adenine dinucleotide) as the coenzyme and nitroblue tetrazolium as the electron acceptor[2]. Polyvinyl alcohol (Cytochemical CO.) in 20% was added in the medium in order to stabilize the tissue[1]. Control sections were incubated in the medium without the substrate or with no NAD. A positive enzymatic activity was recognized as bluish purple deposits of the reduced diformazan granules in the tissue.

The chemical assay of the aforementioned dehydrogenase in the tadpole liver was conducted according to Philpott and Peron[21].

Thirty to sixty mg of the liver tissue of each control and E_2-immersed tadpoles were individually homogenized in ice-cold 0.25 M sucrose, buffered to pH 7.4. The homogenates were incubated in a metabolic shaker at 37C under 95% O_2: 5% CO_2 for 30 minutes. The incubation medium contained pregnenolone, NAD, $NaHCO_3$, KCl and trace amount of [7(n)-^{3}H]-Δ^5-pregnenolone (sp. act. 10.5 Ci per mmole, Radiochemical Centre). At the end of incubation, radioactivity of a small portion of the medium was determined with a Packard Tricarb liquid scintillation spectrometer Model 3320. The remaining medium was centrifuged after reaction with digitonin and the radioactivity of the supernatant was counted. The enzymatic activity was calculated as nM pregnenolone converted to its metabolites by 30 mg liver per 30 minutes. The method takes the advantage of the fact that pregnenolone is precipitated by digitonin while further metabolites which lack the Δ^5-3β-ol configuration are not.

RESULTS

The growth of the tadpoles appeared to be hampered by E_2 treatment. The body weight of the treated animals was about half of that of the controls (Table 1).

TABLE I

Amount of pregnenolone converted by Δ^5-3β-HSD in the tadpole liver

	*n*M of pregnenolone per 30 mg liver per 30 minutes, av±SE	Average final body weight of tadpoles, gm±SE
Control tadpole	21.75±0.92(4)*	3.28±0.33(14)
E_2-treated tadpole	11.35±0.71(6)	1.77±0.19(31)

* Numerals in parentheses represent number of tadpoles.

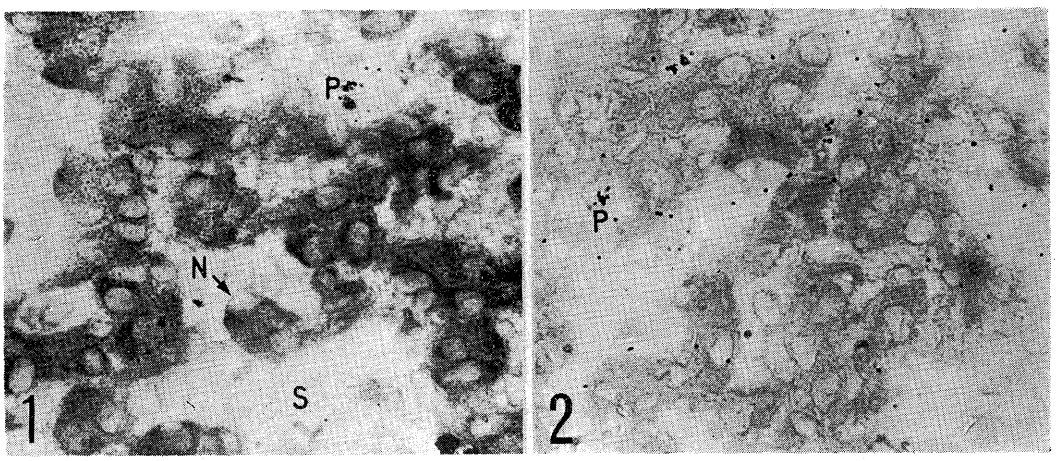
I. Histochemical localization of the enzyme activity

A general histological picture of the tadpole liver shows that the organ is composed mainly of hepatic cells in tubular and plate-like arrangements. In between the tubes and plates there are large sinusoids. Numerous pigment cells and their granules are scattered among the hepatic cells. These histological findings agree essentially with the results observed in livers of frogs[16] and *Xenopus laevis*[27].

Deposition of colored diformazan granules was observed in the cytoplasm of the hepatic cells only. Sections incubated in the medium free of DHEA also gave a positive result but the diformazan granules were less, indicating the presence of a small amount of endogenous substrate in the liver. In NAD free medium the result was negative. When comparison of the enzyme activity in the liver was made between control and experimental tadpoles, it was very obvious that Δ^5-3β-HSD was much reduced by the hormone treatment as shown in Figs. 1 and 2.

II. Biochemical assay of the enzyme activity

The result of the radioactivity count is shown in Table 1. It is clear that the dehydrogenase activity in E_2-treated tadpole livers was



LEGEND OF FIGURES

The 2 figures are sections of tadpole livers prepared histochemically. The dark granules in the cytoplasm of hepatic cells are diformazan deposits while granules outside the cells are pigment granules (P). Note the absence of the enzyme activity in the nucleus (N). S represents hepatic sinosoids. All figures are enlarged 480×

Fig. 1. Control liver showing profound Δ^5-3β-HSD activity.
Fig. 2. E_2-treated liver showing reduced enzyme activity.

reduced to half when compared with that of the controls; the difference was statistically very significant ($p<0.001$).

DISCUSSION

Studies on Δ^5-3β-HSD in the amphibian liver have been very meager. Baillie *et al.*[2] found histochemically no activity in the liver of *Rana temporaria*. This negative finding could be due to technical problems as evidenced by the fact that no activity of this dehydrogenase toward DHEA was noticed in the rat liver by Levy *et al.*[18] and Goldman *et al.*[15]; but later on the same activity was demonstrated in rat[2] and mouse livers (unpublished data of this laboratory).

To our knowledge, no biochemical data concerning this hepatic enzyme in tadpoles are available, but there are some positive findings on the enzymatic activity in rat liver preparations[3,4,10,14].

Therefore, the present finding of the activity of Δ^5-3β-HSD in the tadpole liver is the first report in amphibians. The functional significance of this enzyme in the tadpole liver merits discussion. It may concern steroid metabolism or could be otherwise than endocrinological.

Although the existence of Δ^5-3β-HSD in mammalian liver has been reported, yet the role it plays in steroid metabolism is ambiguous. The organ was proposed as the extra-adrenal site of conversion of Δ^5-3β-steroids to pregnanetriol in the urine of patients of congenital adrenal hyperplasia due to Δ^5-3β-HSD difficiency[6]. Parks *et al.*[20] reported a case of pubertal boy with the genetic defect of Δ^5-3β-HSD in adrenals and testes, and the paradoxical laboratory findings of progesterone in blood and of pregnanetriol, androsterone and etiocholanolone in the urine of the patient were explained by the authors on the basis of possible peripheral conversion of pregnene compounds to pregnane compounds. It was also suggested that this hepatic enzyme could be responsible for peripheral conversion of DHEA to testosterone in rats resulting in virilizing effects on the develop-

ment of the Wolffian duct in spite of the reduction of Δ^5-3β-HSD in the duct by cyanoketone[15].

It seems that liver Δ^5-3β-HSD may be the extra-gonadal and extra-adrenal sites for metabolism of steroids possessing Δ^5-3β-ol structure. Since amphibians and their tadpoles are known to possess steroid hormones comparable to those of mammals[7~9,22~26,28,30] it is apt to think that the tadpole liver may also play a role in steroid metabolism.

On the other hand, it has been recognized that in the rat liver Δ^5-3β-HSD is involved in the biosynthesis of bile acids from cholesterol[3,4]. Since bile secretion is also one of the digestive functions in amphibians, this enzyme may be presumed to perform the same duty in tadpoles.

The present histochemical observation on the reduction of Δ^5-3β-HSD activity by E_2 treatment paralleled closely to the result of radioactivity assay. The former method presented a localized enzyme activity in the cytoplasm of hepatic cells while the assay determination indicated quantitatively how much the enzyme activity was decreased.

It was demonstrated that activity of Δ^5-3β-HSD from the bacteria, *Pseudomonas testosteroni* and in ovaries and adrenals of pregnant rats and fetuses were reduced by E_2 treatment[5,11~13]. These authors held that E_2 produces a stoichiometric and irreversible inhibition on the enzyme like that of a synthetic steroid, cyanoketone.

The same enzyme activity was also decreased by E_2 in tadpole interrenals and ovaries, resulting in disturbance of estrogen synthesis and degeneration of ovaries[17]. Therefore, the reduction of Δ^5-3β-HSD activity in the tadpole liver by E_2 is conceivable.

The reduction of body weight in the tadpoles treated with E_2 may be due to the decreased activity of the hepatic Δ^5-3β-HSD, culminating in interference of bile formation and thus tadpole growth. On the other hand, toxicity of E_2 when delivered in large dosage could also be responsible for the reduction of the body weight as suggested by Goldman[12] for his rat experiments.

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雌性二醇對蝌蚪肝中 Δ^5-3β-羥基固醇類去氫酶之影響

許織雲　許劉信

將牛蛙蝌蚪飼養於每公升含 2 mg 雌性二醇 (17β-Estradiol) 之水中，經七個月後犧牲之；取其肝臟做組織化學及生化研究，以觀察 Δ^5-3β-羥基固醇類去氫酶受雌性二醇的影響情形。結果顯示：無論用組織化學法或生化法，該酶活性均受到雌性二醇的抑制。本文並就功能方面討論該酶與膽酸合成及肝中固醇類荷爾蒙代謝間的關係。